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Chromosome analysis of chironomids (Diptera: Chironomidae) – obligatory miners of freshwater sponges (Porifera)

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Abstract. Karyotypic characteristics of the chironomids *Demeijerea rufipes* (Linnaeus, 1761) and *Xenochironomus* sp., obligatory miners of freshwater sponges, are considered. The karyotype of *Xenochironomus* sp. is described for the first time. It is assumed that the ordered spatial organization of chromosomes of these species, expressed in terms of the presence of chromocentres of different degrees of stability and an almost complete absence of polymorphism for chromosome rearrangements, represents an adaptation to inhabiting host tissues, i.e. to habitat relative constancy.

Key words: *Xenochironomus*, *Demeijerea*, karyotype, chromocentre.

Introduction

The genera *Xenochironomus* Kieffer, 1921 and *Demeijerea* Kruseman, 1933 belong to the tribe Chironomini of the subfamily Chironominae (Chironomidae, Diptera), and their larvae are regarded as obligatory miners of freshwater sponges (Shilova, 1974; Pinder, Reiss, 1983). There is information on the karyotypes of two species of these genera. For *D. rufipes* (Linnaeus, 1761) (2n=6) chromosome arms were denoted (Belyanina, 1993) and for *X. xenolabis* Kieffer, 1916 (2n=8) a cytophotomap of polytene chromosomes was elaborated (Belyanina, Durnova, 2002). For both the species presence of chromocentre in the karyotype was established.

The goal of the present work is studying the karyotype of *Xenochironomus* sp., presumably new to science, morphologically similar to *X. xenolabis*, and obtaining additional information on cytogenetic characteristics of *D. rufipes*.

Material and methods

Material was collected in water-bodies of the Saratov Region (Table). Larvae were extracted from the body of the sponge *Spongilla lacustris* Linnaeus, 1758 when its tissues were dissected in a Petri dish. IV-instar larvae were fixed in mixture of 96% ethanol and glacial acetic acid (3:1). Identification of species was based on larval morphology (Shilova, 1974; Pankratova, 1983; Pinder, Reiss, 1983). Preparations of polytene chromosomes from salivary gland cells were made by ethyl-orcein technique (Dyomin, Shobanov, 1990). The study of the morphology of larvae and polytene chromosomes was carried out under a microscope with magnifications of 15×40 and 15×90. Microphotographs of polytene chro-

Table. Collecting data.

Species	Localities	Date of collection	Number of investigated individuals
Xenochironomus sp.	Tereshka River, Saratov Region, Russia	12.07.05.	53
Demeijerea rufipes L.	Volga River near Engels, Saratov Region, Russia	18.08.05.	15

mosomes were obtained using a Practica DC 44 digital photocamera.

Results

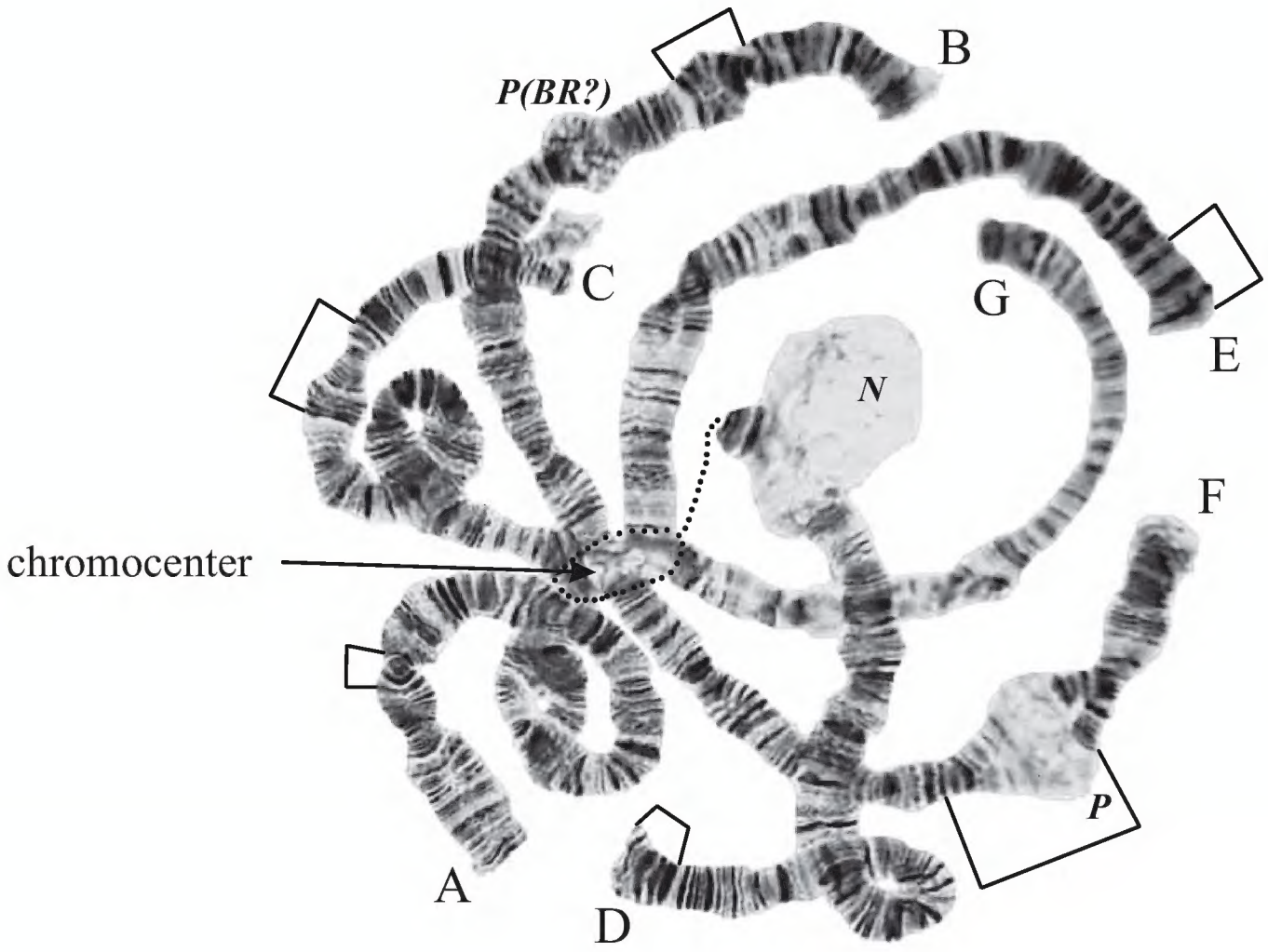
Xenochironomus sp. (Fig. 1, a-c; 2, b, d, f). In the karyotype there are seven chromosome arms that are joined in a chromocentre. Based on the odd number of arms it is supposed that at least one pair of chromosomes is acrocentric. It has not been established which arms comprise other chromosomes, therefore the morphology of chromosomes and their number remains unclear. However, we have revealed small sites similar in structure to the previously studied chromosomes of *X. xenolabis* (Belyanina, Durnova, 2002), and on the basis of this finding chromosome arms of *Xenochironomus* sp. have been marked.

A description of the karyotypes is given below, based on comparison with the karyotype of *X. xenolabis*. In arm A there is a small constriction similar to that in section 15 of arm A in *X. xenolabis* (xenA). In arm B there is a puff (possibly Balbiani ring), similar in morphology to the active region in the section 22 xenB. Additionally, arm B is marked by a thickening restricted on the two sides by a small constriction as in the sections 23-25 xenB. Arm C contains a constriction similar to constriction in section 18 xenC. Arm D contains one nucleolus (N), situated close to the chromocentre; the dark discs on the distal part of this arm are similar to discs in sections 27-28 xenD. In arm E no common markers for the two species have been revealed; in *Xenochironomus* sp. this arm is marked by three dark disks on the distal end. In arm F in *Xenochironomus* sp. a large puff and three distinct discs near it as in the sections 5-7 xenF are situated. Arm G in the karyotypes of both the species is the shortest and thinnest.

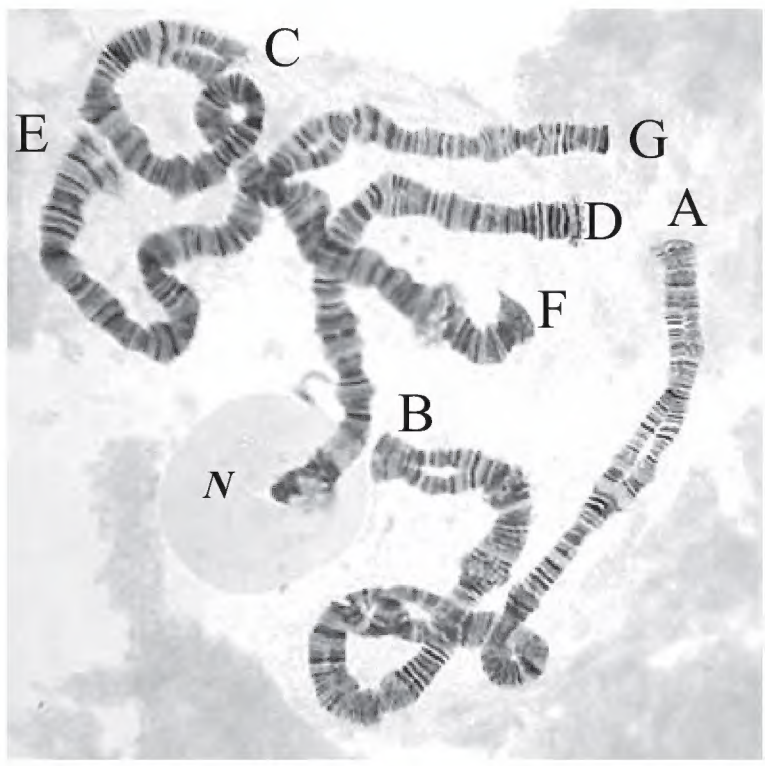
When cytological slides were prepared using the method of squashing the chromocentre was retained in the majority of cells of *Xenochironomus* sp., but in approximately 20% of cases arm D containing a nucleolus separated from it (Fig. 1, a). Sometimes different combinations of two arms (Fig. 1, b, c) occurred with approximately similar frequency, and the most preferable associations of arms have not been revealed.

In few larvae a small heterozygous inversion was revealed in arm A close to chromocentre. In arms B, C, and G there are areas with partially destroyed conjugation of homologues, which is probably a consequence of heterozygous microrearrangements.

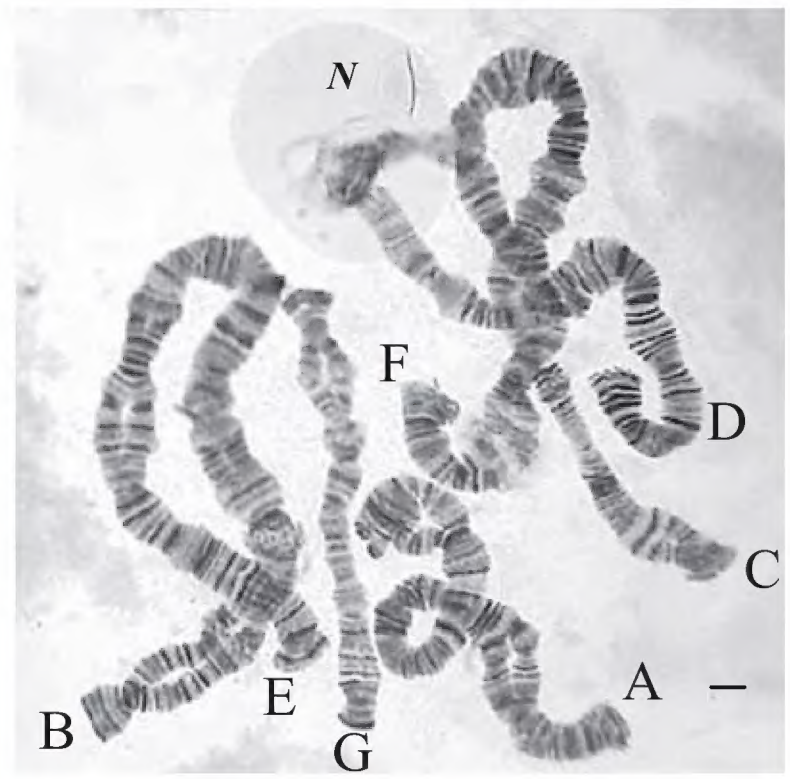
In spite of the great similarity of larvae of *Xenochironomus* sp. and *X. xenolabis*, some morphological differences between them have



a



b



c

Fig. 1, a-c. Karyotype of *Xenochironomus* sp. **a** - common chromocentre is not destroyed. **b**, **c** - chromocentre is destroyed; **b** - combination of arms AB and arm D are separated from chromocentre; **c** - combination of arms BE and arms A and G are separated from chromocentre. A, B, C, D, E, F, G - chromosome arms. Marker features of chromosome arms are designated by square brackets. N - nucleolus, p - puff, BR - Balbiani ring. Bar=10 μm.

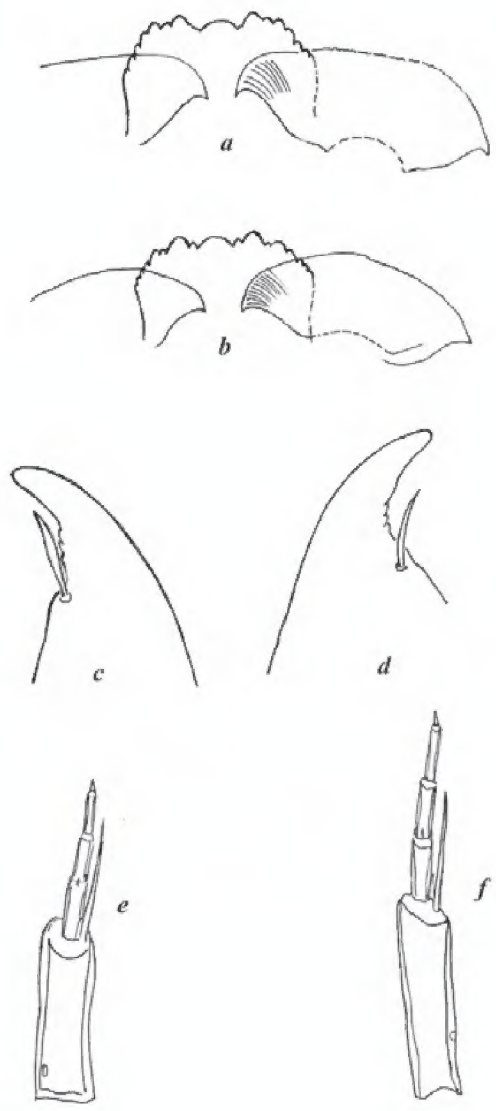


Fig. 2, a-f. Morphological characteristics of larvae of *X. xenolabis* (**a**, **c**, **e**) and *Xenochironomus* sp. (**b**, **d**, **f**). **a**, **b** - mentum and ventromental plate. **c**, **d** - mandible. **e**, **f** - antennae.

been revealed. In *Xenochironomus* sp. close to the large median denticle of mentum usually there are small denticles (Fig. 2, b), exterior ones according to some authors (Pinder, Reiss, 1983; Makarchenko, 2006) but first lateral ones according to other authors (Shilova, 1974; Pankratova, 1983). In *X. xenolabis* these denticles in the majority of cases are completely reduced (Fig. 2, a). In *X. xenolabis* the seta beneath the denticles of the mandible is slightly extended in the middle part (Fig. 2, c), whereas in *Xenochironomus* sp. it is thinner, smoothly narrowing towards the apex (Fig. 2, d). In *Xenochironomus* sp. the sensilla of the antenna nearly reaches the end of the third segment (Fig. 2, f), whereas in *X. xenolabis* it terminates in the lower half of the fourth segment (Fig. 2, e).

Demeijerea rufipes (Fig. 3, a, b). In the karyotype there are 6 chromosome arms joined in a chromocentre. It has not been established what particular arms comprise individual chromosomes, therefore the morphology and chromosome number have not been clarified. The polytene chromosome disc pattern conforms to the earlier published data (Belyanina, 1993).

In the present study measurement has been carried out for the first time; and designation of chromosome arms has been made beginning with the longest arm: rufA (IR)> rufB (IIIR)>rufC (IIR)>rufD (IL)≥rufE (IIL)>rufF (IIIL). When cytological slides were prepared with the squashing method the chromocentre was retained in the majority of cells (Fig. 3, a), but in some cases (approximately 10-15% of cells) its structure was disturbed and arms were separated, lying either separately, or in the form of associations, mostly in combinations B+D (Fig. 3, b), A+F, C+E (not shown). Chromosome rearrangements have not been recorded.

Discussion

The shared characteristic of the karyotypes of *Xenochironomus* sp. and *D. rufipes* is the ordered arrangement of chromosomes in salivary gland cells caused by the presence of a single chromocentre of differing degrees of stability (more stable in *Xenochironomus* sp. as compared to *D. rufipes*). This phenomenon was earlier described in *X. xenolabis* (Belyanina, Durnova, 2002). For *D. rufipes* preferable associations of chromosome arms (AF, BD, CE) have been established that were observed when the single chromocentre was destroyed. It is assumed that *D. rufipes* has 2n=6 (Belyanina, 1993), but the study of the metaphase plate is required to determine the exact chromosome number of this species. In *Xenochironomus* sp. and *X. xenolabis* mechani-

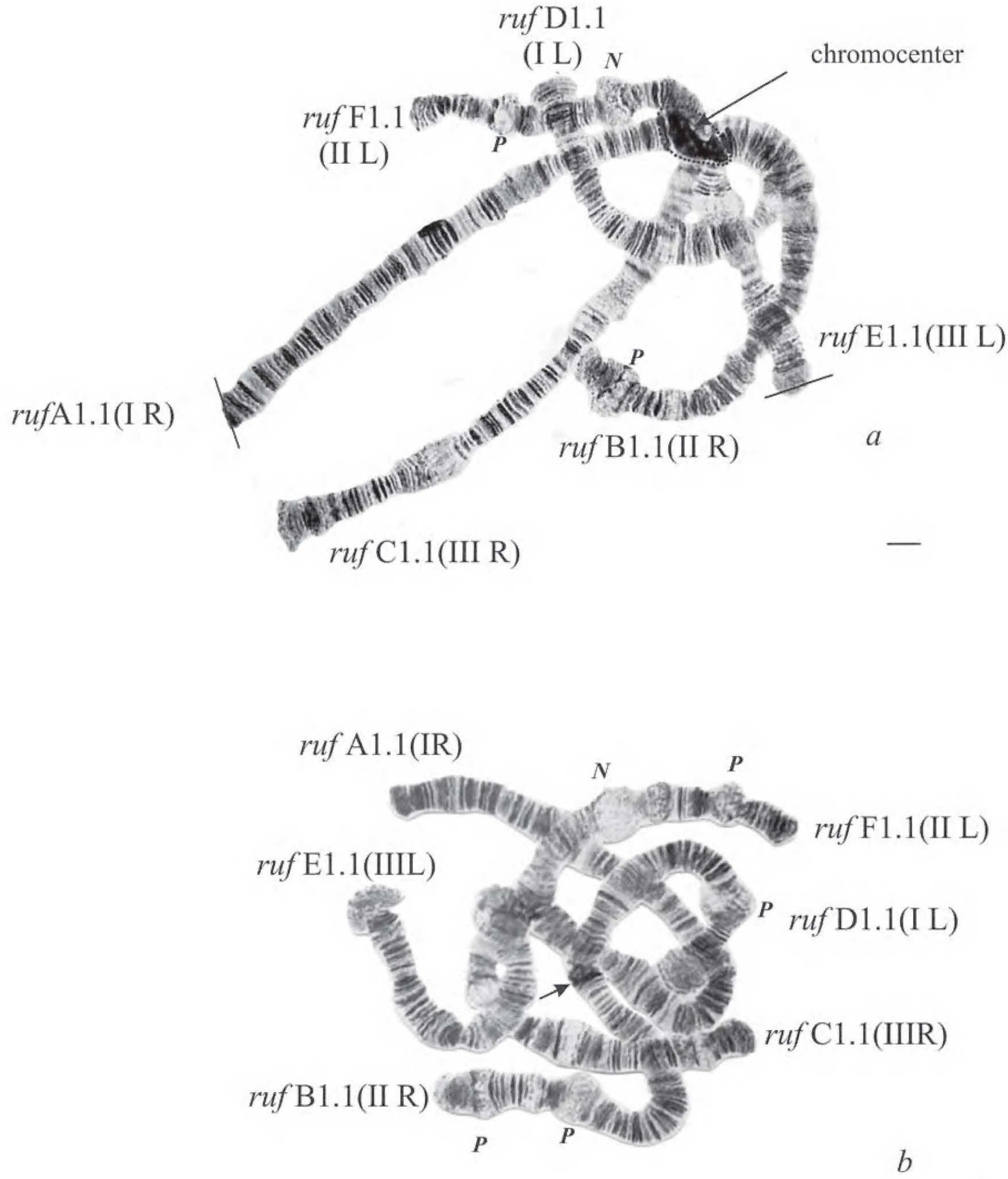


Fig. 3, a-b. Karyotype of *D. rufipes*. **a** - chromocentre is not destroyed. **b** - chromocentre is destroyed. Denomination of chromosome arms: rufA (IR), rufB (IIR), rufC (IIIR), rufD (IL), rufE (IIIL), rufF (IIL); in brackets – denomination of chromosome arms after Belyanina (1993). The other designations as in Fig. 1. Bar=10 μm.

cal destruction of the chromocentre in rare cases led to accidental combinations of two arms. Analysis of metaphase chromosomes of *X. xenolabis* has shown that this species has 2n=8 (Belyanina, Durnova, 2002). It can be assumed that closely related *Xenochironomus* sp. also has 2n=8, but this supposition needs corroboration. The polytene chromosomes of *Xenochironomus* sp. as compared to *X. xenolabis* are characterized by a specific pattern of discs and localization of active regions.

Associations of non-homologous chromosomes and the presence of chromocentres have also been described in other chironomid species belonging to different subfamilies. Thus, in subfamilies Tanypodinae, Prodiamesinae and Orthocladiinae this phenomenon was noted in *Clinotanypus nervosus* (Meigen, 1818) (Belyanina, Sigareva, 1981), *Prodiamesa olivacea* (Meigen, 1818) (Petrova, Chubareva, 1978) and *Cricotopus algarum* (Kieffer, 1911) (Michailova, 1989) respectively. In the subfamily Chironominae a chromocentre occurs very rarely; in *Chironomus melanotus* Keyl, 1961, *Ch. cucini* Webb, 1969 (Wülker, Butler, 1983) and *Ch. pilicornis* Fabricius, 1794 (Kiknadze et al., 2002) the chromocentre is facultative, and in *Lipiniella arenicola* Shilova, 1961 (Kiknadze et al., 1991) and *Parachironomus parilis* (Walker, 1856) (Michailova, 1989) it is permanent.

The functional role of spatial arrangement of chromosomes in nucleus has not been established (Zhimulev, 1992). It is known, however, that the presence of a chromocentre leads to ordered spatial arrangement of chromosomes in the interphase nucleus and probably to restriction of variation of their structure (Stegniy, 1993). Joining of centromeres into a single chromocentre is regarded as a primitive state and disappearance of the chromocentre as an evolutionary tendency characteristic of the family Chironomidae and other Diptera (Stegniy, 1993; Shobanov, 2002).

The species considered in the present article are narrowly specialized and they are bound by close biotic relations with freshwater sponges. It can be suggested that the presence of a chromocentre testifies to a relative evolutionary primitiveness of these species. The absence (or low frequency) of chromosomal rearrangements is apparently connected with the relatively constant habitat conditions of larvae inhabiting sponge tissues.

At the time of the present only one species of the genus *Xenochironomus* – *X. xenolabis*, which has been reported also for Russia, is known from the Holarctic (Zorina, 2006). This study performed shows that *Xenochironomus* sp. has notable differences from *X. xenolabis* in both karyotype and larval morphology. This allows the assumption that *Xenochironomus* sp. is a new species for Russia, and possibly also to science. However, this could be stated more convincingly after all the stages of metamorphosis, and primarily adult male morphology are studied.

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